Reference: *Biol. Bull.*, **140**: 15–27. (February, 1971)

LUMINESCENCE CONTROL IN *PORICHTHYS* (TELEOSTEI): EXCITATION OF ISOLATED PHOTOPHORES [1]

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All luminous fish are marine and the great majority of them live in deep waters. Consequently they are generally in poor condition after capture and not amenable to physiological study. An exception is the batrachoid teleost *Porichthys* (midshipman), a mid-water luminescent fish which moves inshore during the breeding season (Arora, 1948). It is well endowed with photophores, luminous spots on head and trunk, each containing a mass of photocytes (luminescent cells), nerve endings, a reflector, pigment-backing and a lens (Nicol, 1957; Strum, 1969). *Porichthys* is a hardy fish, easy to capture in good condition and to keep alive in captivity. These qualities make it favorable material for physiological investigations and, indeed, of the very few investigations of fish photophore physiology, the most extensive studies have been performed on this fish. It has been shown that *Porichthys* luminescence can be initiated by electrical stimulation of the whole animal (Greene, 1899), by injection of adrenaline (Green and Greene, 1924), or by electrical stimulation of the exposed spinal cord (Nicol, 1957). From these observations and a few others on deep sea fishes (Ohshima, 1911; Harvey, 1931), it is believed that photophores are either under hormonal control or nervous control, or that hormonal control is primary and nervous control secondary (Nicol, 1967). However, nothing is known either about the physiological properties of the photophore itself or about the manner in which the presumed control mechanisms initiate luminescence.

This work is devoted to the study of isolated *Porichthys* photophores subjected to electrical stimulation in order to analyze more precisely the excitation mechanism by elimination of all influences of the internal environment and to establish optimum conditions for further studies.

Methods

1. *Maintaining fish and photophore preparations*

Specimens of *Porichthys myriaster* and *P. notatus* were collected in shallow water along the Pacific coast near Santa Barbara by divers or by dredging. After capture, they were placed in large aquaria with slowly running sea water. Before dissection, fish were removed from the aquarium and anaesthetized with quinaldine solution (25% in acetone) diluted to a final concentration of 3 ml per liter sea water, a concentration that induces anaesthesia after 3 to 5 minutes. Photo-

[1] Supported by U. S. Office of Naval Research and by a NATO post-doctoral grant.

[2] Chargé de recherches au F.N.R.S. (Belgium).

phores were then detached with as little surrounding tissue as possible. These experiments were all done on photophores from the anal, gastric and mandibular regions, as named by Greene (1899). All these respond similarly to electrical stimulation, although those of the mandibular region are the most consistently and readily excitable.

The isolated photophores, which are 1–2 mm in diameter, were placed in a small vessel filled with air-saturated Young's (1933) marine teleost saline (NaCl, 13.5 g; KCL, 0.6 g; $CaCl_2$, 0.25 g; $MgCl_2$, 0.35 g per liter solution). In order to adjust the pH to 7.4, the value recorded from blood samples, 1 mM $NaHCO_3$ was added.

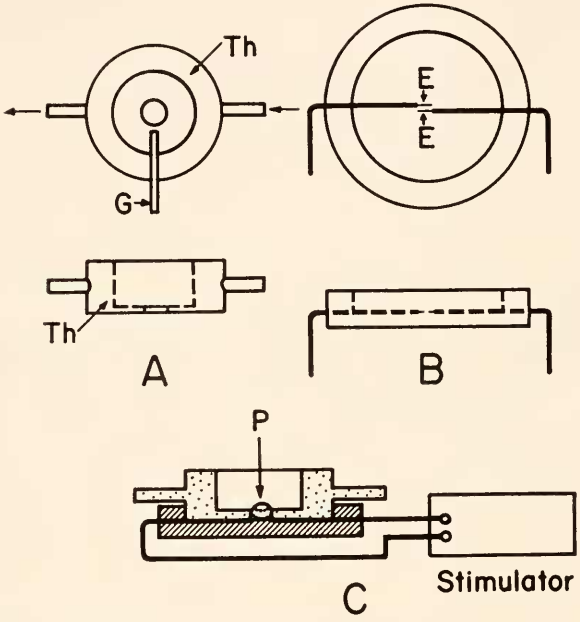

FIGURE 1. Recording chamber; A. thermostatted container (Th) through which a hypodermic needle (G) conducts gas to the specimen; B. plastic chamber bearing stimulating electrodes (E); C. the two chamber assemblies fitted together with a photophore (P) in position.

After dissection, fish were returned to the aquarium where they recovered after 15 to 25 minutes and could subsequently be used many times. Four specimens from which about 50 photophores were removed in this manner survived for 3 to 4 months. For the experiments reported here, 9 male specimens were used, of which 8 were specimens of *P. myriaster*. Their length ranged between 20 and 40 cm and their weights between 180 and 340 g.

2. Recording chamber

After being held for 10 minutes in saline at 20° C, the photophore was placed with dermal surface in contact with two platinum stimulating electrodes (diameter

100 μ) arranged in a chamber as shown in Figure 1. This is a double chamber: part A is a hollow cylinder 1 cm high, 2 cm inner diameter and 3.5 cm outer diameter, with a 4 mm diameter hole drilled in the base. A thermostated fluid flows through the space between the inner and outer walls. Part B is a lucite cylinder hollowed out in order to contain part A to a depth of 5 mm. Two grooves in the floor hold the electrodes. When the two parts are fitted together as shown in Figure 1C, the photophore protrudes into part A through the hole in its base and tissues surrounding the light organ are pressed between parts A and B, preventing movement of the specimen.

3. Recording light emission

A photomultiplier tube (RCA Type 1P21) was covered by a light-tight tube with an opening at the level of the photocathode. A ring was attached to the opening to accept the photophore chamber (Fig. 2). Thus the specimen could be

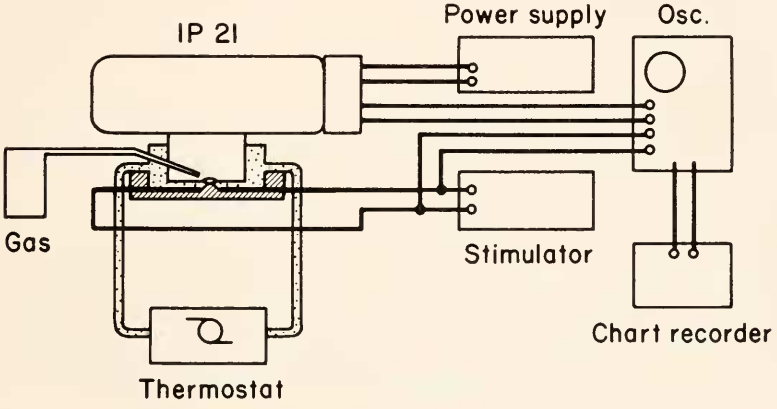

FIGURE 2. Diagram of apparatus used in detecting and recording luminescent responses to electrical stimulation.

enclosed with the light organ facing the photocathode at a distance of 15 mm. Oxygen, air, or nitrogen was passed into this enclosure through a small needle crossing obliquely the inner and outer walls of part A. Except for the nitrogen, the gases were humidified before reaching the photophore, which was stimulated in the gas phase.

Stimulating electrodes contacting the dermal surface of the photophore were connected to a Grass stimulator delivering square pulses. A stimulus artifact was registered by one of the two channels of a Tektronix 502A oscilloscope and one channel of a chart recorder (Offner Dynograph) connected in series to the oscilloscope. The luminescent response from the specimen activated the photomultiplier tube whose electrical signal was recorded on the second channel of the scope and the chart recorder. The photomultiplier was operated at 935 v delivered across a linear voltage divider. A quantitative estimate of light production was obtained with a standard light source in the same location as the experimental tissues. The standard was a disc covered with a C^{14} impregnated

phosphor. The apparatus was generally used so that a 1 mm deflection of the chart recorder trace corresponded to 10^7 quanta/sec. Preparation resistance fluctuated between 400 kΩ and 900 kΩ.

Results

1. *Types of light responses*

(*a.*) *Responses to single stimuli.* After 10 minutes in saline saturated with atmospheric oxygen, no light response was obtained with single stimuli applied

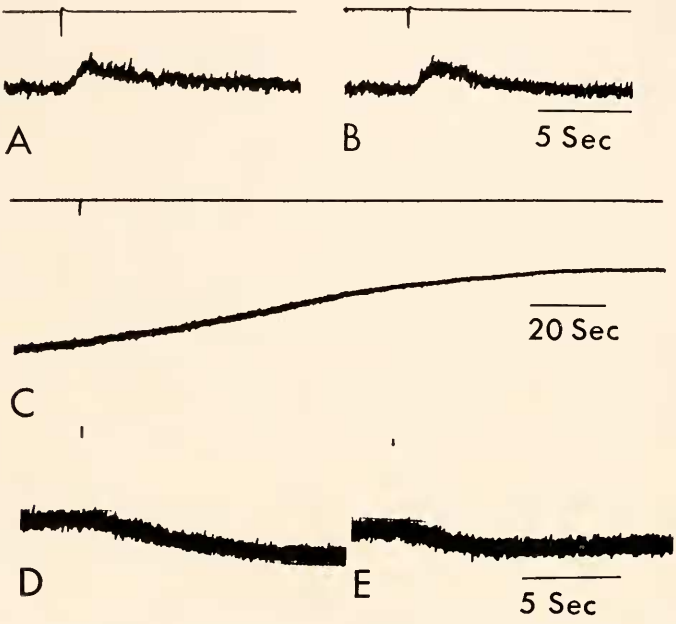

Figure 3. Light production of photophores responding to single stimuli; A and B with microelectrodes in photophore (A, 20 msec., 13 v; B, 20 msec, 10 v); C. a photophore stimulated with external electrodes (20 msec, 10 v) after 2 hours in saline; D and E. diminution of spontaneous glowing associated with stimulation by single pulses from external electrodes (7 msec, 10 v). A, B, D, E recorded at 5 mm/sec, C at 1 mm/sec.

by external electrodes. With microelectrodes (2–5 μ tip) inserted into the light organ itself, responses were obtained with single stimuli of 7 msec duration at 10 to 15 v. Figure 3 shows a recording of light emission after a 13 v, 20 msec stimulus (A) and a 10 v, 20 msec stimulus (B). The emission latency time, *i.e.,* the period between the stimulus and the first recorded light emission, ranged from 100 to 200 msec; the maximum light peak occurred after 0.6 to 1.0 sec and light disappeared after 4 to 8 sec.

The effect of a single stimulus, either *via* external or microelectrodes, was different for photophores which were bathed for longer than one hour in saline after dissection. Some specimens thus treated remained dark when placed in the chamber, while others spontaneously glowed. Those in the first category,

when stimulated with single pulses, glowed for 20 to 30 minutes (Fig. 3C). Usually the photophore did not respond to the next stimulus, presumably a manifestation of injury. In spontaneously glowing specimens, light emission was reduced in a transitory way after a single stimulus. As shown in Figure 3D and E, the amplitude of the reduction depended on the strength of the stimulus: the effect was more evident at 10 v (D) than at 6 v (E).

It was generally found that all isolated photophores began spontaneously glowing after 2 to 3 hours in saline, and after a long-lasting light emission (30 to 60 minutes) became unexcitable electrically or chemically, undoubtedly consequent upon tissue deterioration.

(*b.*) *Responses to multiple stimuli.* In these experiments, electrical stimulation was delivered by external electrodes. Figure 4A shows a recording of typical light emission from a specimen stimulated with multiple stimuli, namely a 20 sec

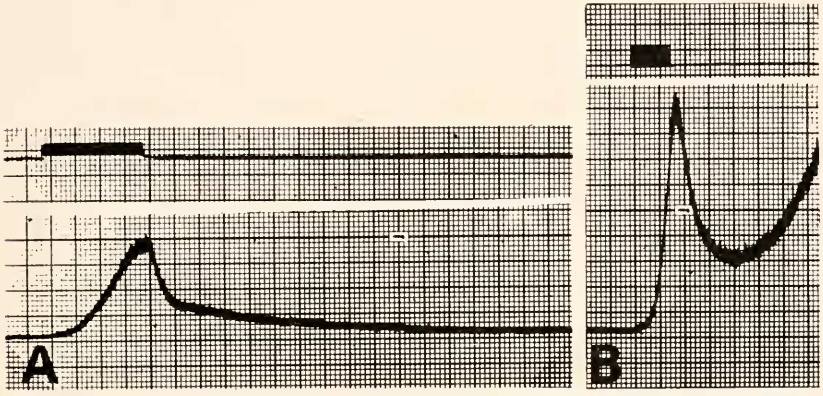

FIGURE 4. Photophore responses to repetitive electrical excitation; A. 20 second stimulus train at 10/sec, 7 msec, 8 v; B. 8 second train at 10/sec, 20 msec, 8 V; in both, photophore in oxygen atmosphere at 20° C; recording at 1 mm/sec.

train of 7 msec, 8 v stimuli at a frequency of 10/sec (20°)C). A characteristically long emission latency time always occurred between the first stimulus and the first detectable light emission. Likewise, there was substantial delay, on the order of a second, between the last stimulus and the beginning of light extinction. Light emission, which was maximal after 21 sec, terminated about 130 sec after the end of stimulation.

For purposes of comparison with the effects of single shocks, it is evident that approximately 75 times more light is generated by the preparation shown in Figure 4A than is generated by the preparation of 3A.

With stimuli longer than 10 msec, spontaneous glowing occurred after the light response to electrical stimulation. The intensity of this glowing was about twice that of the initial response. The example shown in Figure 4B was induced by a 10 v, 20 msec, 10/sec stimulation for 8 sec. After such behavior, the photophore usually no longer responded to electrical, chemical, or mechanical stimulation. Consequently, in the experiments reported in this paper, 7 msec

stimuli were used, permitting application of high frequency stimulation without spontaneous glowing which we assume to represent damage.

Repetitive bouts of stimulation initially cause an increase in response magnitude followed subsequently by diminished responses. This is illustrated in Figure 5 where emission rates (black circles) and the corresponding emission latency times (open circles) of 10 successive responses are plotted. At 10/sec responses

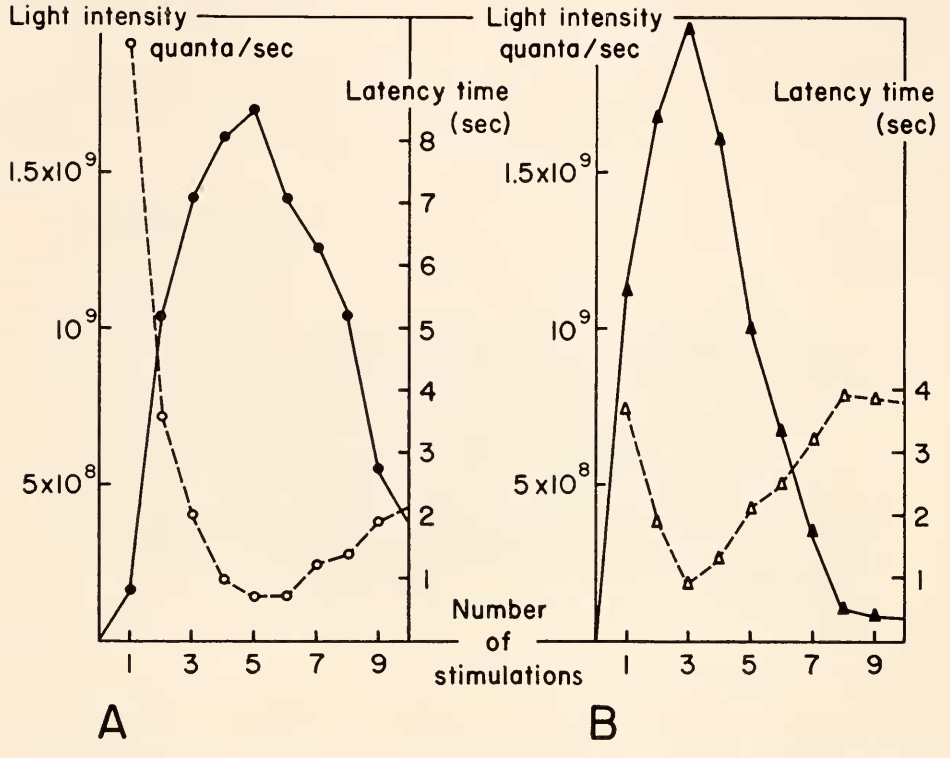

FIGURE 5. Emission latency (right ordinates) and rate of light emission (left ordinates) recorded during 10 stimulations of 20 seconds administered at 3 min intervals: A. 10/sec stimulation frequency, latency (○), emission rate (⊙); B. 20/sec stimulation frequency, latency (△), emission rate (▲).

became maximal at the fifth and subsequently diminished so that the response to the tenth stimulation was only 24% of the maximal. At 20/sec (Fig. 5B) the time course was faster and the response was maximal during the third stimulation while during the tenth the response was only 4% of maximum. The emission latency time was long during the first response, shortest when the response was maximal and thereafter increased again as the light response decreased in magnitude. The response was facilitated by stimulus train repetition, but after several trains fatigue appeared and light emission was reduced.

2. Factors influencing light emission

(*a.*) *Electrical stimulation.* With external electrodes a minimal frequency of 4–5/sec was required to produce a response. The effects of higher frequencies were studied as follows: Each of a series of six photophores was stimulated for 20 seconds at 5, 10 and 20/sec and constant voltage (10 v). Because repetition facilitates the light response, the effects of different frequencies are influenced by the order of application. It was, therefore, necessary to randomize the treatments according to a latin square design (Cochran and Cox, 1957). Figure 6

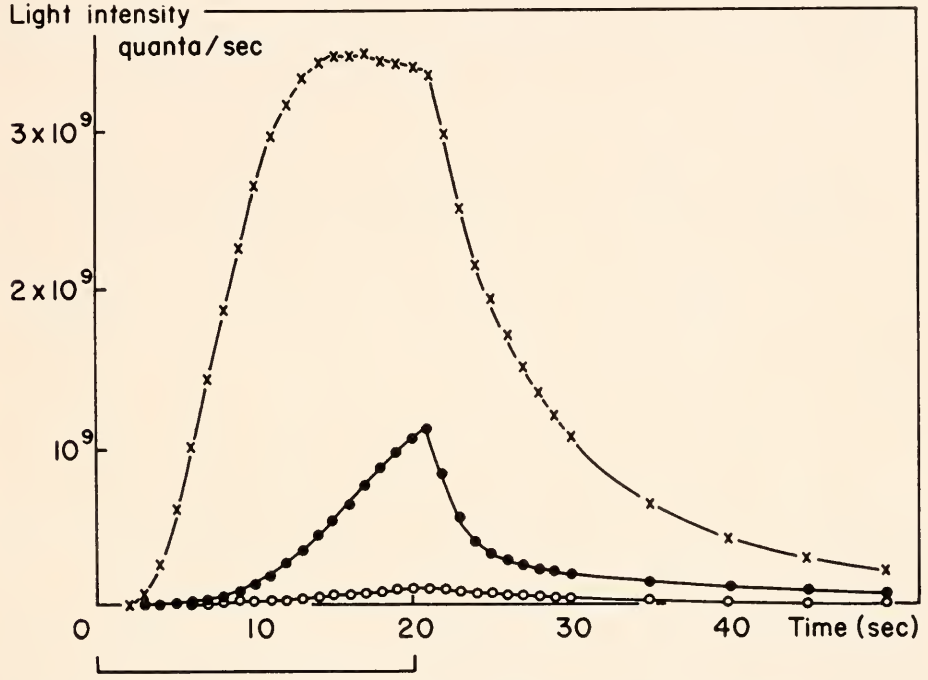

FIGURE 6. Light response of a photophore stimulated at three frequencies: 5/sec (○), 10/sec (●) and 20/sec (×). Horizontal bar indicates stimulus duration of 20 seconds. Prepared in 100% oxygen.

shows the time course of the mean curves calculated for the three treatments. The magnitude of the light response increased with frequency: at 5 and 10/sec, light production increased during the entire stimulation period, while at 20/sec, the curve reached a plateau after about 15 seconds. At 5/sec the response was characterized by a long emission latency time (8.98 ± 1.18 sec, standard error of mean) and a low rate of light emission (7.3×10^7 quanta/sec at the peak). With an increase of frequency to 10/sec, emission latency time decreased significantly ($P < 0.01$) to 3.70 ± 1.18 sec and the rate of light emission was about 15 times greater (103.1×10^7 quanta/sec). At 20/sec, the latency time was shorter than that calculated for 10/sec (1.37 ± 1.8 sec) but the difference is not significant. On the other hand, the maximum rate of light emission was obviously

increased (335×10^7 quanta/sec). The half-extinction time; *i.e.*, the time necessary for the light to decline to half the peak value was 25.0 sec (5/sec); 23.9 sec (10/sec) and 28.3 (20/sec). With a standard error of the mean of ± 0.7 sec, only the half-extinction time recorded at 20/sec was significantly different ($P < 0.05$) from those at 5 and 10/sec.

In all experiments, extinction was complete by 150 seconds after the end of stimulation. Total light produced during this period of time was obtained by integration: 207×10^7 quanta for a photophore receiving 100 stimuli (5/sec over 20 seconds), 1570×10^7 quanta for 200 stimuli (10/sec over 20 seconds) and 7490×10^7 quanta for 400 stimuli (20/sec over 20 seconds), standard error of mean 500×10^7 quanta. Thus at 20/sec, a photophore emitted about 5 times more light than at 10/sec. With higher stimulus voltages the shape and the magnitude of the light emission was not different from that recorded at 10 v.

TABLE I

Effects of saline pH on Porichthys *luminescence* [*Mean values calculated from 12 photophores stimulated in oxygen at 20° C (10 v, 10/sec, 20 seconds); each photophore treated at each pH in "cross-over" sequence; s.e.m. = standard error of mean*]

pH	Emission latency time seconds	Maximum light emission 10^7 quanta/sec	Extinction latency time seconds	Light production over 150 sec 10^7 quanta/sec
7.4	1.91	92.4	0.9	1,523.1
5.6	2.44	36.0	1.0	606.3
s.e.m.	± 0.23	± 12.1	± 0.1	± 179

The effects described above are observed only in cases where specimens are stimulated under specific physiological conditions; the photophore must be held in saline at pH 7.4 after dissection and be stimulated in oxygen at 20° C. The light response is modified when these conditions are not fulfilled.

(*b.*) *Saline pH.* Without adding 1 mM $NaHCO_3$, the pH of the saline was 5.6. When a photophore was stimulated after 10 minutes in this saline, it produced less light than after 10 minutes in the saline at pH 7.4 (Table I). At pH 5.6 the mean peak rate of light emission was only 36×10^7 quanta/sec while it was 92.4×10^7 quanta/sec at pH 7.4 ($n = 12$; standard error of mean $\pm 12.1 \times 10^7$ quanta/sec). The total amount of light produced was 2.5 times greater at pH 7.4 than at pH 5.6. As shown in Table I, neither emission nor extinction latency times were significantly changed by the change of pH.

(*c.*) *Oxygen pressure.* A reduction of the concentration of oxygen from 100% to atmospheric in the experimental chamber brought about a decrease in the light response: by 30% for a 10 v stimulation and by 50% for an 8 v stimulation. The other response characteristics did not change significantly.

When oxygen was removed and replaced by nitrogen for a period of between two and five minutes, the specimen was still excitable but the response was modified (Fig. 7). This figure shows the time course of the mean curve calculated for 8 photophores, each one stimulated, according to latin-square design, in oxygen and nitrogen at 8 and 10 v. The effects of anaerobiosis were an increase

of emission latency time and a reduction of peak light production. The extinction phenomenon was not affected at 10 v but was significantly slowed at 8 v. Prolonged anaerobiosis was necessary to prevent the light response of a photophore. In all instances when a specimen was exposed to nitrogen for more than 5 minutes no light response was obtained on stimulation. However, it is significant to note that the stimulation itself changed the state of the light organ, for after stimulating in nitrogen readmission of oxygen led to spontaneous light emission lasting 2 to 5 minutes.

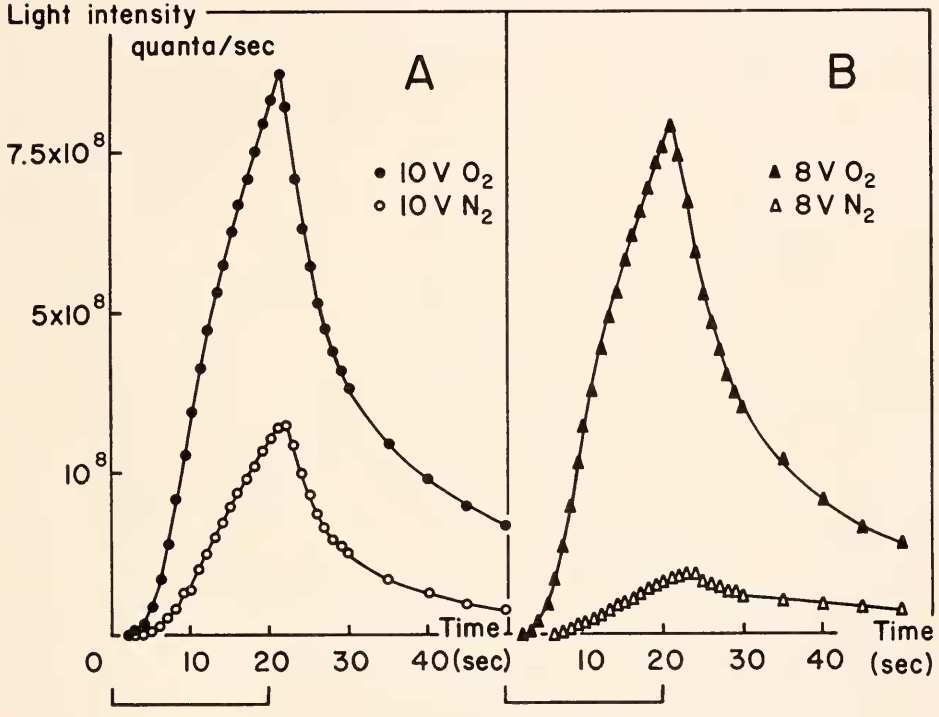

FIGURE 7. Mean curves of light production during stimulation at 10/sec for 20 seconds in oxygen and in nitrogen; A. 10 v; B. 8 v, temperature 20° C.

(*d.*) *Temperature.* Light emission was reversibly affected by temperature changes between 10 and 30° C, with the effects appearing 30 seconds after the temperature change. Figure 8 shows the time course of the mean curves of light emission at 10, 20, and 30° C ($n = 6$, 10 v, 10/sec, 20 seconds stimulation) with the three treatments randomized. Each specimen was held for 3 minutes at the experimental temperature in the thermostatted chamber before stimulation. The response was maximal at 20° C and minimal at 10° C. Table II summarizes the characteristics of the mean response were similar during and after the excitation period. At 10° C, the response was somewhat different; light appeared later [emission latency time significantly longer ($P < 0.01$)] and the magnitude

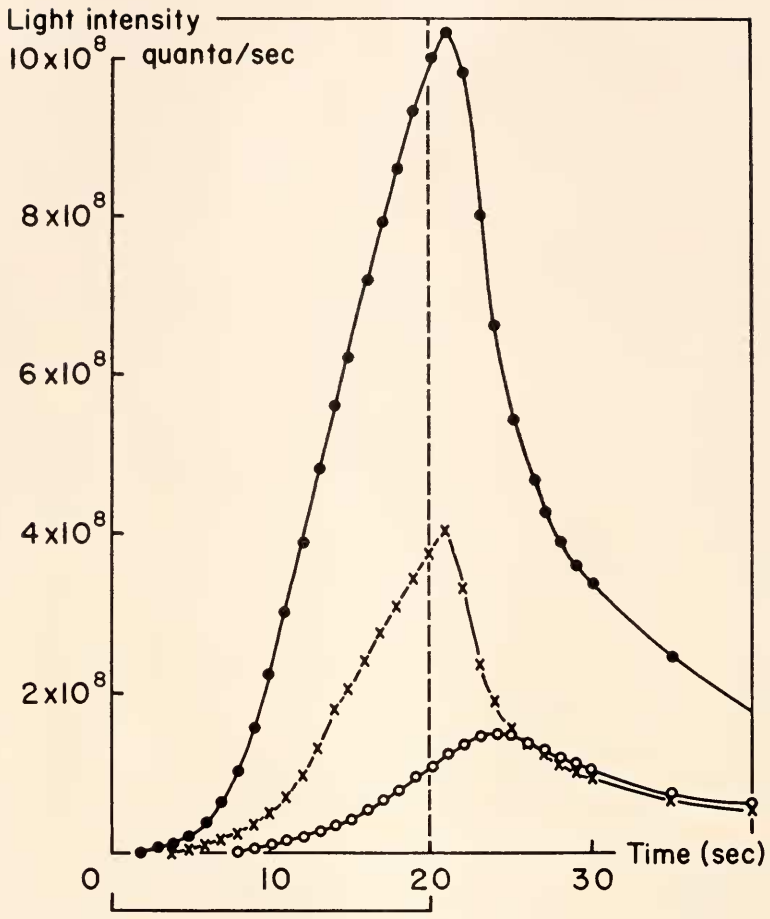

FIGURE 8. Mean curves of light production in response to stimulation at 10/sec for 20 seconds at 10° C (○), 20° C (●), and 30° C (×) in oxygen.

of the response was still increasing 5 seconds after the end of stimulation. Extinction was also significantly slowed ($P < 0.01$).

TABLE II

Temperature influence on Porichthys *luminescence* ($n = 6$; *in oxygen*; *10 v, 10/sec, 20 seconds*; *s.e.m. = std. error of mean*)

Temperature °C	Emission latency time seconds	Maximum light emission 10^7 quanta/sec	Extinction latency time seconds	Half extinction time seconds
10	8.97	14.7	5.24	38.83
20	2.72	105.6	1.17	25.35
30	4.29	40.4	0.89	24.48
s.e.m.	±0.87	±10.5	±0.23	±1.98

Discussion

Isolated *Porichthys* photophores produce light upon electrical stimulation. Their response is characterized by a relatively long emission latency time, never less than one second, and by progressive increase in the rate of light emission during stimulation. If the emission latency time is the time required to gain a threshold at which the light response is triggered, then threshold is reached about three times at late at 5/sec as at 10 or 20/sec. However, since the total number of stimuli delivered during this latent period is the same for the three test frequencies, it is suggested that the effects induced by each stimulus are additive and that light appears when this sum reaches a threshold. As might be expected, if the interval between two stimuli is too long, no light emission is triggered, as seen at low frequency (2/sec). Similarly, Nicol (1957) observed that stimulation of the spinal cord of *Porichthys* with a train of pulses at frequencies below 3/sec was unable to induce luminescence.

In Nicol's experiments latency time varied from 7 to 15 seconds at 4/sec. Strum (1969), who studied nerve fibers of photophores by electron microscopy, suggests that the response time measured by Nicol corresponds largely to the time taken by the nervous influx to reach the photophore from the CNS. According to our data on the isolated photophore, the emission latency time varies from 5 to 19 seconds for a 5/sec stimulation. Thus photophores stimulated either *in situ* through the spinal cord or after isolation, at similar frequencies show a similar latency time. It is possible that, in our experiments, we have stimulated nervous elements isolated along with the light organ. If the electrical current stimulates nerves in our experiments, the latency time cannot correspond to nerve conduction time but rather might be associated with processes occurring at the level of "nerve-photocyte" junctions. Strum (1969) describes nerve-photocyte junctions having a specialized structure: non-myelinated nerve fibers enter the light organ from a deep subdermal plexus (Whitear, 1952) but do not make direct contact with the photocytes, but rather with a "basal lamella" surrounding the posterior regions of the photocytes. These are separated from the lamella by a sinus which appears to be empty. Finally, it should be recalled that when microelectrodes are implanted in the photophore, there is a response to a single pulse with a much shorter latency time, about 100 to 200 msec. The different properties of excitability and the short latency time thus apparent might imply that, in this case, the photogenic cells are directly stimulated. This is borne out by experiments in progress that show the minimal latency for light generation by photophores *in vivo* is in excess of 1 second for electrical excitation of the photophore nerve and not less than 5 seconds for nor-epinephrine injection within a millimeter of the photophore.

Once triggered, light production increases progressively during stimulation. According to Nicol (1957) a photophore contains about 600 to 700 photogenic cells. This progressive increase might correspond either to a recruitment of cells or, assuming all the cells are simultaneously stimulated, to an increase of their light emission. Our results do not enable us to distinguish between these two hypotheses.

The magnitude of the light response is particularly dependent on oxygen pressure and temperature. Light production on stimulation is diminished by a reduction of the oxygen pressure and it is suppressed when the photocyte is exposed to N_2 for more than five minutes. However, it is most interesting that

anaerobiosis does not suppress the excitability of the light organ because on re-admitting O_2 after stimulation in nitrogen, a spontaneous light response occurs. Stimulation may liberate reactants involved in the light reactions, but these possibly cannot react until oxygen is supplied. Certainly oxygen seems to be essential for these reactions to take place. Cormier, Crane, and Nakano (1967) extracted luciferin and luciferase from *Porichthys* photophores, and these were shown to react and produce light when in the presence of oxygen. The presence of numerous mitochondria in the photocytes (Strum, 1969) suggests that intensive oxidative reactions might take place in these cells.

The magnitude of the luminescent response is greatest around 20° C. The temperature coefficient (Q_{10}) calculated by comparing total light production at 10 and 20° C, is estimated as 4.5. The temperature coefficient of the light reaction itself is probably lower; according to Ulidenfriend (1962) the Q_{10} of chemoluminescent reactions is not greater than 1.5. Indeed the coefficient of the luminous system of the crustacean *Cypridina hilgendorfii in vitro* is estimated at 1.14 (Chase and Lorentz, 1945). There is no information concerning the effects of temperature on the isolated luminous system of *Porichthys*. The high value we calculate might be explained by assuming the presence of a series of reactions preceding the luminous reactions; depolarization phenomena, for example.

It is surprising that the optimal temperature for light production is around 20° C, when the temperature of the *Porichthys* mid-water habitat is around 10° C. Perhaps an explanation lies in the fact that during the spawning season, the fish seeks warmer water. Crane (1965) observed the courtship display of *Porichthys* in the aquarium and observed a well-defined associated pattern of luminescence. Since bioluminescence plays a role in the courtship pattern of *Porichthys* and since isolated photophores produce little light at low temperature (10° C), it is conceivable that the mechanisms of luminescence are better adapted to the breeding environment rather than to its usual habitat.

From our results, the light energy produced by a photophore may be roughly estimated, assuming the wavelengths of light from an intact photophore and from the luminous system *in vitro* are similar, approximately 460 mμ (Cromier, Crane and Nakano, 1967). For minimal stimulation at 10° C, the peak of light production is estimated as 6.5×10^{-1} microwatt; at 20° C, 4.6×10^{-4} microwatt. For a maximal stimulation at 20/sec and 20° C oxygen, the output is 1.4×10^{-3} microwatt. These computations are rendered highly approximate if only owing to the difficulty of ascertaining the geometry of light emission and the possibility of light absorption by associated tissues and especially chromatophores.

We are most indebted to Mr. Jules Crane, Dr. Richard Ibara and Mr. M. S. Trinkle for assistance in obtaining *Porichthys* and for their most helpful advice and assistance.

Summary

Luminescene of isolated photophores of midshipman fish, *Porichthys myriaster* and *P. notatus,* was studied as a function of electrical stimulus characteristics, temperature, oxygen concentration and pH. Single stimuli delivered into the

photophore *via* microelectrode induced luminescence with a minimum latency of 100 msec. Externally applied stimuli were ineffective at rates of less than 2/sec and at 5/sec latency was between 5 and 19 seconds. These values suggest the possibility of both direct and indirect photophore excitability. Oxygen is essential for luminescence, but spontaneous occurrence of luminescence after electrical stimulation under nitrogen suggests that certain elements of the excitation-luminescence sequence are relatively insensitive to anaerobiosis. The temperature optimum is at about 20° C and the Q_{10} is 4.5. Total light produced was greater at pH 7.4 than at 5.6. Maximal light production is estimated at 1.4×10^{-3} microwatt per photophore.

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